EVALUATION OF SERUM ANTICARDIOLIPIN ANTIBODY IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT DIABETES MELLITUS

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BRANCH II – PERIODONTICS

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CERTIFICATE

This is to certify that **Dr. J. PIRANITHA**, Postgraduate student in the Department of Periodontics, J.K.K. Nattraja Dental College and Hospital, Komarapalayam – 638183 has done this dissertation titled **" EVALUATION OF SERUM ANTICARDIOLIPIN ANTIBODY IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT DIABETES MELLITUS"** under our direct guidance and supervision during her Postgraduate study period 2010 – 2013.

This dissertation is submitted to **THE TAMILNADU Dr. M. G. R MEDICAL UNIVERSITY, CHENNAI,** in partial fulfillment of the Degree of **MASTER OF DENTAL SURGERY, BRANCH II - PERIODONTICS.**

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ABSTRACT:

EVALUATION OF SERUM ANTICARDIOLIPIN ANTIBODY IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT DIABETES MELLITUS

AIMS AND OBJECTIVES:

The aim of this present study was to evaluate serum anticardiolipin antibody (ACA) IgG and IgM in Chronic Periodontitis (CP) patients, Healthy controls, Type 2 Diabetes Mellitus and Chronic Periodontitis with Type 2 Diabetes Mellitus patients and to find out whether CP has its role to influence ACA levels in Type 2 Diabetes Mellitus patients.

MATERIALS AND METHODS:

Venous blood samples were collected from four groups in a clean and dry tubes. Serum was separated and stored until analysis. Anticardiolipin antibody ELISA kit (Enzyme Linked Immuno Sorbent Assay) was used to assess IgG and IgM ACA levels. The results were subjected to statistical analysis.

RESULTS:

Cardiolipin is one of the phospholipid present in the inner mitochondrial membrane. The results showed that ACA IgG levels were more in Chronic Periodontitis with Type 2 Diabetes Mellitus group compared to other three groups. The ACA IgM levels were low compared to other three groups.

CONCLUSION:

The results proved that CP has role to increase the ACA levels in Diabetes group and periodontal health is important to maintain diabetic patient's health. Therefore, within the limitations of this study it can be concluded that CP is one of the risk factor for Diabetes Mellitus.

Keywords: Anticardiolipin antibody, Type 2 Diabetes Mellitus, Chronic Periodontitis.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action or both[1]. It is not only metabolic disorder but also a vascular disease.[1]

The endothelial lining of blood vessels which maintains the homeostasis induced by different stimuli is destructed in diabetes due to hyperglycemia, hyperinsulinemia, dyslipidemia and thrombotic state.[1] Mitochondrial destruction of pancreatic islet occurs leading to β cell failure and results in the evolution of type 2 diabetes mellitus.[2]

These mitochondrial destruction also leads to the formation of anticardiolipin antibodies (ACA). Antibodies to cardiolipin are termed as anti-cardiolipin antibodies (ACA) which are the subset of anti-phospholipid antibodies.[4] Cardiolipin is the major component of myocardium involved in oxidative respiration and electron transport chain.[5]

Anti-phospholipid antibodies are classified as anti-cardiolipin antibodies, Lupus anticoagulant, beta 2 glycoprotein 1 dependent and independent IgG, IgM, IgA autoantibodies.[4]

Anti-cardiolipin antibodies are elevated in patients with Systemic Lupus Erythematosus,[6] Recurrent Abortions,[7] Ischemic Heart Disease,[8] Atherosclerosis,[9] Stroke,[10] Buerger disease,[11] Acute Myocardial Infarction,[12] Diabetes,[13] Cardiovascular disease,[14] Pulmonary disease, Rheumatoid Arthritis (RA)[15] and Periodontitis.[3]

The risk of Cardiovascular diseases such as atherosclerosis increases in the presence of Diabetes Mellitus (DM) due to the deposition of reactive advanced glycation end products.[16]

Various studies have been reported linking diabetes and periodontitis suggesting a bidirectional relationship and periodontitis as "sixth" complication of diabetes.

Periodontitis is an inflammatory disease of tooth-supporting structures that leads to the destruction of connective tissue, loss of periodontal attachment and resorption of alveolar bone.[3] It is initiated and perpetuated by a group of predominantly Gram-negative anaerobic microorganisms.

Lipopolysaccharide (LPS) the outer membrane component of Gram-negative microorganisms is a highly potent stimulus that induces the expression of a series of cytokines and inflammatory mediators from host cells and causes injury to periodontal tissues. The microorganisms also activate cellular and humoral immune systems systemically. This increases the risk of systemic diseases namely Diabetes mellitus,[3] Cardiovascular diseases, [14] Pulmonary diseases, Preterm-low-birth weight infants.

The activated humoral immunity releases various antibodies against its antigens. These antibodies can be used as a biomarker to predict the progression of various systemic and periodontal diseases.[17]

One such biomarker is the antibodies directed against cardiolipin, a phospholipid (diphosphatidyl glycerol) present in the inner mitochondrial membrane.[17]

Anti-cardiolipin antibodies are elevated in periodontitis patients due to the molecular mimicry between the peptide sequence of arginine-gingipain protease of the periodontal pathogen *Porphyromonas gingivalis* and beta 2 glycoprotein 1 (β_2GP1).[18] β_2GP1 is a natural anticoagulant present in endothelium which prevents pathological prothrombotic reactions initiated by platelets or endothelial cells. ACA target these β_2GP1 and causes arterial and venous thrombosis leading to various systemic sequelae of periodontitis like cardiovascular diseases.[11]

The risk for cardiovascular disease in diabetes and periodontitis is more prevalent nowadays. In the light of the facts mentioned above, we can presume that ACA can be evaluated to assess the cardiovascular risks in patients with periodontitis and diabetes.

To assess the risk of cardiovascular disease or atherosclerosis in diabetic and Chronic periodontitis patients, the anti-cardiolipin antibodies can be evaluated. Therefore, the aim of this present study is to evaluate serum anti-cardiolipin antibody levels in Chronic periodontitis and Diabetes Mellitus and to correlate whether Chronic periodontitis has its effect to influence the anti-cardiolipin antibody levels in diabetic patients.

AIMS AND OBJECTIVES

The aim of this present study is to evaluate serum anti-cardiolipin antibody levels in four different groups using Enzyme Linked Immuno Sorbent Assay (ELISA) method. The results were subjected to statistical analysis.

The four groups are:

Group A: Healthy controls

Group B: Chronic Periodontitis

Group C: Type 2 diabetes mellitus

Group D: Chronic Periodontitis with Type 2 diabetes mellitus

REVIEW OF LITERATURE

Cardiolipin is a phospholipid present in the inner mitochondrial membrane, where it constitutes about 20% of the total lipid composition. The name cardiolipin is derived from the fact that it was first found in animal hearts.

Anti-cardiolipin antibodies are antibodies often directed against cardiolipin and found in several diseases including Antiphospholipid Syndrome,[19] Behcet's Syndrome, Idiopathic spontaneous abortions, Systemic Lupus Erythematosus (SLE),[4] Diabetes Mellitus,[11] and Periodontitis.[3]

Anti-cardiolipin antibodies are classified into three types[10]

1. IgG
2. IgM
3. IgA

Diabetes mellitus is a chronic metabolic and vascular disease.[1] The endothelial lining of blood vessels which maintains the homeostasis induced by different stimuli is destructed in diabetes.[1] In Diabetes mitochondrial destruction occurs.[2] Therefore, elicits antibody response to cardiolipin.

In periodontitis patients anti-cardiolipin antibodies are elevated.[17] Periodontitis and Diabetes Mellitus are interrelated to each other.

DIABETES MELLITUS IN GENERAL

Deedwania PC (1997)[1] suggested that endothelial dysfunction is one of the earliest steps in the process of atherosclerosis and vascular disease. Hyperglycemia, hyperinsulinemia, dyslipidemia, and prothrombotic state are responsible for vascular damage in diabetes. Therefore, diabetic patients are more prone for atherosclerosis and other vascular diseases.

Hoogwerf BJ (2005)[20] in his review about the complications of diabetes found that the risk for atherosclerosis is increased in diabetes. The risk for neuropathic and microvascular complications is related to duration and degree of hyperglycemia. Retinopathy leads to blindness and the nephropathy is the leading cause of the need for renal replacement therapy in all diabetic patients.

Mitra A (2008)[21] analysed whether diabetes may be an outcome of stress. In diabetes the "fight or flight" response does not work well. Insulin is not always able to let the extra energy into the cells. The glucose piles up during stress because the insulin is not sufficient to control the blood glucose elevated by the various growth hormones.

Clayton W, Elasy TA (2009)[22] studied the pathophysiology of diabetic foot ulcers. The causes are peripheral neuropathy and ischemia from peripheral vascular disease. The chemical conversion of glucose results in a depletion of nicotinamide adenine dinucleotide phosphate stores leading to increase in oxidative stress on the nerve cell and an increase in vasoconstriction. Therefore, leads to the pathogenesis of diabetic foot ulcers.

Basta G, Schmidt M (2010)[16] suggested that the formation of advanced glycation end products (AGEs) is an important biochemical abnormality that accompanies diabetes mellitus and likely, inflammation in general. AGEs potentially modulate initiating steps in atherogenesis involving blood vessel wall interactions, triggering an inflammatory-proliferative process.

Yamagishi SI, Matsui T (2010)[23] analysed the pathophysiologic role of AGE-RAGE-oxidative stress in diabetic nephropathy. Inhibition of the AGE formation or the blockade of RAGE-downstream pathways will be a promising therapeutic strategy for the treatment of diabetic patients with nephropathy.

Ma ZA, Zhao Z, and Turk J (2012)[2] identified that mitochondrial dysfunction is a central contributor to β- cell failure in the evolution of type 2 DM. Reactive oxygen species (ROS) produced by the β- cell mitochondria as a result of metabolic stress, activates several stress-response pathways. Therefore, in diabetes mitochondrial destruction occurs which leads to alterations in mitochondrial phospholipids.

Chen Y, Feng B, Li X et al. (2012)[24] found that microparticles (MPs) are small membrane vesicles shed from the plasma membrane surface and express a panel of phospholipids and proteins specific to the cells from which they are derived. MPs are associated with several cardiovascular complications. As multifunction biomarkers, they may contribute to the pathogenesis of diabetes associated vascular diseases.

ANTI-CARDIOLIPIN ANTIBODIES IN GENERAL

Young CGM, Loizou S and Walport MJ (1989)[19] measured the clinical, serological and haematological features of 20 primary APS patients. The clinical features are similar to that of patients with raised antiphospholipid antibodies. The authors concluded that "primary anti-phospholipid syndrome" can occur as a distinct entity.

Patrick H, Simpson RJ, Chesterman CN and Krilis SA (1990)[25] analysed the binding of antiphospholipid antibodies to CL. This binding requires the presence of β_2 glycoprotein I as a cofactor. β_2GPI exerts multiple inhibitory effects on the coagulation pathway and platelet aggregation.

Roubey RA (1994)[26] termed anti-cardiolipin antibodies as antibodies detected in solid phase immunoassays, typically ELISAs, in which in the putative antigen is cardiolipin dried onto a microtiter plate. They are heterogenous, and include both antibodies to cardiolipin and cardiolipin-bound proteins.

Cuadrado MJ, Mujic F, Munoz E et al. (1997)[27] determined whether thrombocytopenia is associated with APS. There was no significant association between thrombocytopenia and clinical or serological manifestations of APS. They are two different clinical entities need to be considered while treating.

Schwarzenbacher R, Zeth K, Diederichs K et al. (1999)[28] studied the crystal structure of β_2GPI, a cofactor for phospholipid binding. The structure comprises of four complement control protein modules and a folding fifth C-terminal domain arranged as beads on a string to form elongated J shaped molecule.

Yanez A, Cedillo L, Neyrolles O et al. (1999)[29] defined antiphospholipid syndrome as a hemocytopenic and vasoocclusive disorder with resultant production of antibodies against phospholipids. The authors found that *Mycoplasma pneumonia*-induced respiratory disease produced antibodies against cardiolipin.

Deguchi H, Fernandez JA, Hackeng TM et al. (2000)[30] defined ACA as a diagnostic for phospholipid antibody syndrome which is associated with increased risks of venous and arterial thrombosis. They showed that epitopes of APS could induce CL or oxidized CL in lipoproteins or in complexes with plasma proteins or with platelet or endothelial surface proteins.

Blank M, Krause I, Fridkin M (2002)[31] identified a hexapeptide (TLRVYK) which was recognized by pathogenic anti-β_2GPI monoclonal antibodies. The authors concluded that bacterial peptides homologous with β_2GPI induces β_2GPI antibodies.

Karvonen J, Paivansalo M, Kesaniemi YA, Horkko S (2003)[32] found that IgM autoantibodies to malondialdehyde-modified LDL (MDA-LDL) have an inverse association with carotid atherosclerosis. The levels of LDL enhances atherogenesis by different mechanisms, which in turn complicates the risk of cardiovascular accidents.

Schlame M (2008)[33] found that CL is a minor component of bacterial and mitochondrial membranes. A sound knowledge about CL will give idea about Barth syndrome, cardiac myopathies and metabolic syndromes. Therefore, CL is important in mitochondrial organization.

Ortana E, Cappozzi A, Colasanti T et al. (2010)[34] studied the new antigenic target for APS patients. The authors found that vimentin act as a cofactor in seronegative APS patients. Therefore, suggested that vimentin act as a good tool for diagnosis.

Osman C, Voelker DR, and Langer T (2011)[5] defined mitochondria as dynamic organelles whose functional integrity requires a coordinated supply of proteins and phospholipids. Phospholipids like CL affect the stability and catalytic activity of mitochondrial membrane proteins. This has become the therapeutic target area nowadays for many diseases which affects mitochondria like diabetes mellitus.

Pradeep Kumar, Panishankar (2011)[4] quantitatively analyzed IgG and IgM anti-CL antibodies in 40 chronic periodontitis patients. Subjects with severe periodontitis exhibited marked increase in ACA IgG and IgM compared to other groups. A positive correlation existed between mean clinical attachment loss and IgG and IgM values. Severe periodontitis subjects are more prone to systemic problems mainly coronary heart disease.

ANTICARDIOLIPIN ANTIBODIES AND SYSTEMIC DISEASES

Out HK, Groot PD, Hasselaar P, Vliet MV and Derksen R (1989)[6] examined systemic lupus erythematosus (SLE) prospectively. The authors found that ACA levels are elevated in SLE patients. Those patients are associated with recurrent or isolated thrombotic stroke in association with ACA.

Coull BM, Bourdette DN, Goodnight SH et al. (1987)[35] found that ACA are not only associated with autoimmune diseases but with infections as acute phase reactants. Understanding the precise role of ACA in the pathogenesis of vascular thromboses is important to find the mechanism of stroke.

Seriolo B, Accardo S, Garnero A et al. (1999)[15] investigated the levels of protein C, S in rheumatoid arthritis (RA) patients with and without CL positivity. The authors found that ACA positivity and lower levels of protein S in RA patients may represent one of the risk factors for thrombotic events. Lower levels of free protein S in ACA positivity patients was involved in the pathogenesis of thrombotic events in SLE and autoimmune diseases.

Kalra S, Tuli A, Goyal U et al. (2002)[7] estimated the prevalence of ACA IgM in patients of first trimester recurrent abortion and also the association between the two. 80% of patients with raised ACA antibody IgM had other infections like toxoplasmosis, tuberculosis. They concluded that ACA antibody was not associated with first trimester recurrent abortions.

Levine JS, Branch DW, Rauch J (2002)[9] reviewed about the antiphospholipid syndrome (APS). They found that activation of endothelial cells and oxidant mediated injury of endothelium and modulation of proteins involved in the coagulation leads to APS. Thrombosis occurs within both venous and arterial beds.

Loizou S, Singh S, Wypkema E et al. (2003)[36] investigated IgG, IgM, and IgA, antiphospholipid antibodies (aPL), against CL, β2GPI, and prothrombin in black South African patients with infectious disease. Anti-prothrombin antibodies are more elevated in those patients with systemic diseases than leprosy who had elevated anti-β_2GPI.

Nojima J, Masuda Y, Iwatani Y et al. (2008)[8] evaluated 155 patients with SLE. Patients diagnosed with ASO had higher ACA/ β_2GPI levels. The presence of ACA/ β_2GPI contributes to the risk of development of ASO, which may represent an important mechanism for the pathogenesis of IHD in patients with SLE.

Edwards CJ, syddall H, Jameson K et al. (2008)[37] measured the ACA in general population. Infections in the childhood influence the ACA levels in the adult due to the development of immune system.

Chen YW, Nagasawa, Ushida Y et al. (2009)[10] assessed serum ACA, anti-TLRVYK, anti-TLRIYT antibodies in 19 Buerger disease patients. Periodontopathic bacteria may serve as exogenous antigens that stimulate the ACA antibody production through molecular mimicry between the bacterial peptides and a host plasma protein.

Zachman DK, Chicco AJ, McCune SA et al. (2010)[38] found that cardiac CL is essential for energetic. CL physically associates with cytochrome C oxidase. The authors developed a new method to isolate and culture CL and found that calcium independent phospholipase A_2 is involved in the rat heart failure.

Skare TL (2011)[39] studied the ACA levels in leg ulcer patients. They found that ACA are elevated in patients with venous and diabetic ulcers and not in arterial ulcer patients. Therefore, clinical characteristics of ulcers do not identify ACA positivity in diabetic patients.

ANTI-CARDIOLIPIN ANTIBODIES AND DIABETES MELLITUS

Cojacaro IM, Cojacaro M, Musuroi C, Botezat M (2003)[40] evaluated the relationship between the prevalence of the IgG anti-cardiolipin antibodies (IgG aCL) and ischemic stroke in patients with diabetes mellitus. The IgG aCL titer did not differ significantly between patients with ischemic stroke and those without stroke. Therefore, the authors concluded that IgG aCL are not a risk factor for ischemic stroke in diabetic patients. IgG aCL indicate inflammatory conditions in these pathologies.

Tarkun I, Hacihanefioglu A et al. (2005)[41] evaluated the association between micro and macrovascular complications of diabetes and aCL and anti-β_2GPI antibodies. There was no association with the microvascular complications and also no significant association between anti- β_2GPI antibodies. The authors concluded that there was no relation between ACA and vascular complications of DM.

Romero J, Rodriguez E (2007)[13] prospectively studied type 2 diabetes mellitus patients and evaluated their IgM and IgG anti-cardiolipin antibodies using ELISA. Low ACA titers may occur in type 2 diabetic patients and not seem to be associated with complicated diabetes mellitus, cardiovascular disease, nephropathy or retinopathy.

Cojocaru M, Popescu L (2008)[42] evaluated IgG, anti-beta 2 glycoprotein (anti-β_2GPI) anti-cardiolipin antibody in type 2 diabetic patients with and without diabetic retinopathy. The results showed that the possible participation of anti-β_2GPI in the pathogenesis of diabetic retinopathy, shifting the homeostatic balance towards a pro-thrombotic state increasing the risk of developing thrombosis.

Ahmed E, Nittyanand S (2009)[43] investigated diabetic patients with and without vascular complications and their ACA levels. The higher prevalence and levels of ACA and circulating immune complexes in patients with vascular complications in their study suggests that these humoral factors might be involved in the vascular complications of type 1 diabetes mellitus.

Shahin M, Amany M, Diasty EL et al. (2009)[44] evaluated 34 diabetics having proliferative diabetic retinopathy (PDR) with high risk criteria (HRC) for ACA. 6 of 34 diabetics with PDR with HRC were positive for ACA. They concluded that presence of ACA may represent an additional risk factor for PDR.

Copetti CE, Perreynoud M, Bisi MC, Staub HL (2012)[45] studied the relationship of ACA with vascular complications of DM. A weak but statistically insignificant association between IgM ACA and diabetic with vasculopathy was found. The authors concluded that there was no association of ACA with vascular events in type 2 diabetes.

PERIODONTITIS IN GENERAL

Armitage GC, Wu F, Wang HY, Sorrell J, Duff GW (2000) [46] classified periodontitis patients based on clinical attachment level as gingivitis, initial periodontitis, moderate periodontitis and severe periodontitis. The authors also found ethinic variation in the polymorphism of interleukin 1A, 1B genotype in Chinese populations.

Hutter JW, Van der Velden U, Varoufaki A et al. (2001)[47] identified lower numbers of erythrocytes and lower levels of hemoglobin in periodontitis patients. Therefore, periodontitis too needs to be considered as a chronic disease which will result in the reduction of number of erythrocytes and hemoglobin leading to anemia.

Joshipura KJ, Rimm EB, Hung HC et al. (2003)[48] found that periodontitis and few teeth are risk factors for ischemic heart disease (IHD), stroke. They conducted a 12 year follow up study and identified that periodontitis patients are at increased risk for IHD and stroke. Patients who had periodontitis as well as few teeth had more risk of developing IHD compared to people with any one disease.

Mercanoglu F et al. (2004)[49]evaluated whether there was endothelial dysfunction in patients with chronic periodontitis. The authors concluded that periodontitis acts as a risk factor for peripheral vascular disease and stroke. The initial periodontal therapy resulted in improvement of endothelial functions. Therefore, periodontal therapy is needed to improve not only periodontal health but also systemic health.

Rufail ML, Schenkein HA, Barbour SE et al. (2005)[50] examined whether the increase of plasma triglycerides in generalized aggressive periodontitis (GAgP) is associated with changes in LDL associated platelet activating factor acetylhydrolase (PAF-AH) activity. The results shown that GAgP patients have more atherogenic lipoprotein profile and lower PAF-AH activity.

Ylostala PV, Jarelin MR, Laitinen J et al. (2006)[51] evaluated the risk factors for CVD. Dental diseases were associated with elevated total cholesterol, low HDL, high LDL and high leukocyte levels. The data proved that gingivitis, dental caries, but not tooth loss, were associated with C-reactive protein levels (CRP).

Fentoglu O, Bozkurt FY (2008)[52] found that periodontal infections causes bacteremia and endotoxeamia and promotes systemic immune and inflammatory reactions. TNF-α and IL-1β induces cytokine production which induces alteration in lipid metabolism. Therefore, the authors concluded that periodontal disease and hyperlipidemia have dual relationship.

Wolff RE, Wolff LF, Bryan S and Michalowicz (2009)[53] assessed the glycosylated hemoglobin levels in periodontitis patients. The authors found a slight elevation in glycosylated hemoglobin. Therefore, the authors concluded that periodontitis is associated with elevated blood glucose in adults without diabetes and may increase one's risk for type 2 DM.

PERIODONTITIS AND DIABETES MELLITUS

Thorstensson H, Kuylenstierna J, Hugoson (1996)[54] evaluated the medical status and complications in relation to periodontal disease experience in insulin-dependent diabetics. The study revealed an association between renal disease, cardiovascular complications and severe periodontitis.

Preshaw PM, Alba (1999)[55] suggested that there exists a clear relationship between the degree of hyperglycemia and severity of periodontitis. Incidences of macroalbuminuria and end-stage renal disease are increased twofold and threefold, respectively, in diabetic individuals having severe periodontitis.

Matthew DC (2000)[56] proved that the diabetes affected all periodontal parameters like bleeding scores, probing depths, loss of attachment and missing tooth. Periodontal therapy influences glycemic control in diabetic patients.

Sadzevicienne R, Paipaliene P (2000)[57] evaluated the periodontal condition in diabetes patients. He concluded that the increase in duration of diabetes and the presence of complications in other organs caused by the disease result in a more severe form of periodontal pathology and also the oral hygiene status affects the development of the inflammatory pathology of periodontal tissues.

Genco J, Grossi SG et al. (2005)[58] proposed a model linking inflammation to obesity, diabetes, and periodontal infections. They evaluated plasma levels of tumour necrosis factor alpha (TNF-α) and its soluble receptors (sTNFα). They concluded that obesity is a significant predictor of periodontal disease and insulin resistance (IR) appears to mediate this relationship.

Nesse W, Linde J (2007)[59] assessed the inflammatory burden using the periodontal inflamed surface area and HbA1C levels in type 2 diabetes. The study concluded a dose response relationship between blood glucose level and the amount of inflamed periodontal tissue in type 2 diabetes. Therefore, as the periodontal inflamed surface area increases the HbA_1C levels also increases.

Engebretson S, Chertog R, Nichols A et al. (2007)[60] evaluated plasma levels of TNF-α in patients with chronic periodontitis and type 2 DM and suggested that elevated circulating TNF-α might contribute to insulin resistance in patients with type 2 DM. TNF was associated with more severe periodontitis and it was likely that periodontitis might influence circulating TNF levels.

Tunes RS, Freitas F and Filho GH (2010)[61] discussed about the impact of periodontal treatment in type 2 DM patients. The authors concluded that cytokine induced low grade inflammation in periodontitis leads to insulin resistance in diabetes. Therefore, periodontal treatment is mandatory to avoid cytokine induced low grade inflammation in diabetic patients.

Malik G, Lehl G, Talwar M et al. (2011)[62] explained the immunobiological association between periodontal disease and diabetes mellitus. TNFα and IL-1 mediated insulin resistance and connective tissue destruction in periodontitis. Periodontal infection mediated synthesis and secretion of cytokine may amplify the AGE mediated cytokine response. Therefore, a dual relationship exists between DM and periodontitis.

Rajhans NS, Kohad RM, Chaudhari VG and Mhaske NH (2011)[63] clinically evaluated the relationship of diabetes mellitus with the periodontal disease. The prevalence of periodontal disease in diabetic patients was 86.8%. The authors concluded that poorer the glycemic control and longer the duration of diabetes the greater will be the prevalence and severity of the periodontitis.

Preshaw PM, Alba AL, Herrera D et al. (2012)[64] suggested that susceptibility to periodontal disease is increased approximately by three fold in people with DM. A clear relationship exists between degree of hyperglycemia and severity of periodontitis. The mechanisms that underpin the links are not completely understood.

PERIODONTITIS AND CARDIOVASCULAR DISEASE

***Jason L, Lavstedt S, Frithiof L et al.* (2001)**[65] studied the relationship between oral health and mortality in CVD. Dental health was found to be a risk indicator of death due to cardiovascular disease, especially in combination with other risk factors like smoking habits.

Lopez, Oyarzun, Naranjo C et al. (2002)[66] evaluated Coronary Heart Disease (CHD) and periodontitis in Chilean adults. Hyperactive monocytes producing high quantities of prothrombotic cytokines when stimulated by lipopolysaccharide (LPS) from Gram negative oral bacteria was found. The results reflected a causal link between periodontal diseases and CHD.

Aigto F, Parkar M, Andreaou G et al. (2004)[67] evaluated the association between periodontitis and atherogenesis and found that periodontal pathogen enters systemic circulation leading to various factors which increases atheroma formation. The authors found that effective control of periodontal infection reduced serum inflammatory markers in small population with periodontitis.

Elter JR, Champagne C, Offenbacher S and Beck JD (2004)[68] evaluated the relationship of tooth loss and periodontitis to prevalent CHD at the atherosclerosis risk in communities (ARIC) study 4. The results proved that when both tooth loss and periodontitis are present CHD is prevalent.

Chun Y, Chun K, Olguin D et al. (2005)[69] evaluated the biological foundation for periodontitis as a potential risk factor for atherosclerosis. *P.g* affected the vessel wall directly or indirectly via inflammatory response, immune responses, hemostasis. Periodontal treatment was expected to reduce the risk of atherosclerosis and CVD.

Leivedaros et al. (2005)[70] studied the measurements of markers of atherosclerosis in periodontitis. It was based on the higher intima thickness (IMT) of internal carotid artery (ICA) and elevated plasma levels of VWF, especially in severe periodontitis compared to subjects without periodontal destruction.

Engebretson SP, Lamster IB, Elkind MSV et al. (2005)[71] assessed whether chronic periodontitis was associated with atherosclerosis using panoramic radiographs. Periodontal bone loss is associated independently with carotid atherosclerosis. Panoramic radiographs may thus help to assess atherosclerosis risk in chronic periodontitis.

Roth GA, Walter FR, Giacona MB et al. (2007)[72] assessed the ability of *P.g* to modulate the properties of endothelial cells. Primary human aortic endothelial cells (HAEC) were infected with *P.g* strain 381. The data demonstrated that live invasive *P.g* 381 elicited a proatherogenic response in HAEC.

Tonetti MS (2009)[73] studied the relationship between periodontitis and risk for atherosclerosis. Periodontal pathogens were able to invade gingival tissues and gain access to systemic circulation. Therefore, periodontitis causes systemic inflammation and endothelial dysfunction.

Segovia M, Reina S, Borda E et al. (2011)[74] evaluated autoantibodies to β_1-adrenoceptor (β_1-AR) in periodontitis patients. The authors found a decrease in heart rate variability (HRV) in periodontitis patients. Anti- β_1-AR IgG exhibited low HRV and agonist like activity.

PERIODONTITIS AND ANTI-CARDIOLIPIN ANTIBODIES

Schenkein HA, Gunsolley JC, Harrison T et al. (1999)[75] hypothetized that phosphorylcholine (PC) is an important oral antigen associated with organism in periodontal microflora and that anti-PC antibody is elevated as a consequence of periodontal disease. Therefore, periodontitis influences the anti-PC levels and in turn leads to systemic complications.

Schenkein HA et al. (2003)[76] evaluated ACA in sera from patients with Periodontitis. The prevalence of ACA was greater in CP and GAgP. There was no prevalence of ACA in localized AgP patients. The study indicated that the antibody specificity of ACA in the sera from periodontitis patients was β_2GPI dependent.

Schenkein HA (2005)[18] found that P.g bears an epitope that is cross-reactive with β_2GPI. High levels of aCL antibodies induces increased uptake of LDL into monocytes. This results in the formation of foam cells during early stages of atheroma formation. Therefore, involved in the pathogenesis of cardiovascular disease sequelae.

Schenkein HA et al. (2007)[77]evaluated ACA and serum adhesion molecule levels in patients with AgP. Soluble intercellular adhesion molecule (sICAM-1), soluble vascular cell adhesion molecule (sVCAM-1) and sE-selectin were measured in subjects with generalized aggressive. sICAM-1 levels are increased compared to other adhesion molecules in AgP.

Turkoglu O, Nezihi Baris. Necil Kutukculer et al. (2008)[3] in their study evaluated serum aCL and ox-LDL in CP patients with essential hypertension. The study concluded that the IgG anti-cardiolipin antibody levels were higher in periodontitis-hypertension group compared to that of other groups. Therefore, CP is involved in the elevation of ACA in Essential Hypertensive patients.

Faghihi SH, Rokn AR, Ebrahimi R (2009)[78] evaluated ACA levels in 51 patients with moderate and advanced chronic peridontitis. Clinical parameters and ACA levels were assessed and there was a positive correlation between ACA levels and periodontal parameters.

Karnoutosos K, Papastergiou P, Stefanidis S, Vakaloudi A (2009)[79] suggested that periodontitis can be added to the risk factors for coronary artery disease and atherosclerosis. Atherosclerosis have inflammatory component with deposition of lipids in the vessel wall. They concluded that oral bacteria from periodontitis can enter systemic circulation resulting in the cytokine production and atherosclerosis and increases aCL antibodies.

Pereira RB, Vasquez EC, Stefanon I and Meyrelles SS (2011)[80] evaluated P.g infection on the apo E deficient mice on the vascular permeability. The study demonstrated that oral *P.g* affects the alpha-adrenoceptor-mediated vascular responsiveness in both healthy and spontaneous atherosclerotic mice, reinforcing the association between periodontitis and cardiovascular diseases.

PERIODONTITIS AND CVD AND ANTICARDIOLIPIN ANTIBODY

Muir KW(1995)[14] found that IgG are elevated in the general population after acute stroke. The ACA levels represent an epiphenomenon of vascular endothelial and endomyocardial damage. Therefore, IgG ACA are involved in patients who had recent events of cardiac attack.

Bili A, Moss AJ, Francis CW, Zareba W et al. (2000)[81] studied the association of ACA with coronary artery disease. They found that elevated IgG ACA and low IgM ACA are independent risk factors for recurrent cardiac events. ACA IgM had inverse association with recurrent cardiac events.

Mohan P, Ashok Kumar S, Geetha S et al. (2011)[11] evaluated 63 patients with acute ST segment elevation myocardial infarction and 63 healthy controls. Patients with hypertension had a significant elevation of aCL antibodies. Therefore, Hypertension was one of the significant risk factor for AMI.

Gunupati S, Chava VK, Phani Krishna (2011)[17]evaluated the effect of phase I periodontal therapy on ACA in patients with Acute Myocardial Infarction (AMI) associated with CP. Clinical parameters were measured in the baseline. Phase I periodontal therapy was performed and all clinical and biochemical parameters were reanalyzed after 1 month which resulted in altered ACA levels. Both IgG and IgM ACA levels were assessed and found that significant difference in IgG levels.

Lei L, Li H, Yan F, Li Y and Xiao Y (2011)[82] treated human monocytes with ox-LDL to induce the formation of foam cells. *P.g* altered atherosclerotic related gene expression in oxidized LDL induced macrophages and foam cells. They concluded that *P.g* LPS appears to be an important factor in the development of atherosclerosis via activation of NFκB pathway.

MATERIALS AND METHODS

For the present cross sectional study, four groups comprising of 68 patients were selected and the levels of serum anticardiolipin antibody IgG and IgM were evaluated. The four study groups were: Group A: Healthy, Group B: type 2 Diabetes Mellitus, Group C: Chronic Periodontitis, Group D: Chronic Periodontitis with type 2 Diabetes Mellitus.

The patients were selected based on Armitage classification (2000)[41] for Chronic Periodontitis and for type 2 Diabetes mellitus based on blood glucose profile with random blood glucose level > 126 mg/dl (WHO) criteria. The patients were selected from diabetes centre and J.K.K. Nattraja Dental College and Hospital, Komarapalayam using the following selection criteria.

Inclusion Criteria: (for all groups)

1) Periodontally and systemically healthy patients.
2) Chronic periodontitis patients who are systemically healthy.
3) Individuals with type 2 diabetes mellitus.
4) Chronic periodontitis with type 2 Diabetes mellitus.
5) Individuals with > 16 teeth present.
6) Mean Clinical attachment level greater than 2.5 mm.
7) Both male and female patients.

Exclusion Criteria:

1) Patients with systemic illness other than type 2 diabetes mellitus.
2) Subjects with vascular complications of diabetes mellitus.
3) Subjects who underwent periodontal treatment during the last 6 months period.

4) Subjects under antibiotic coverage within the past 6 months.

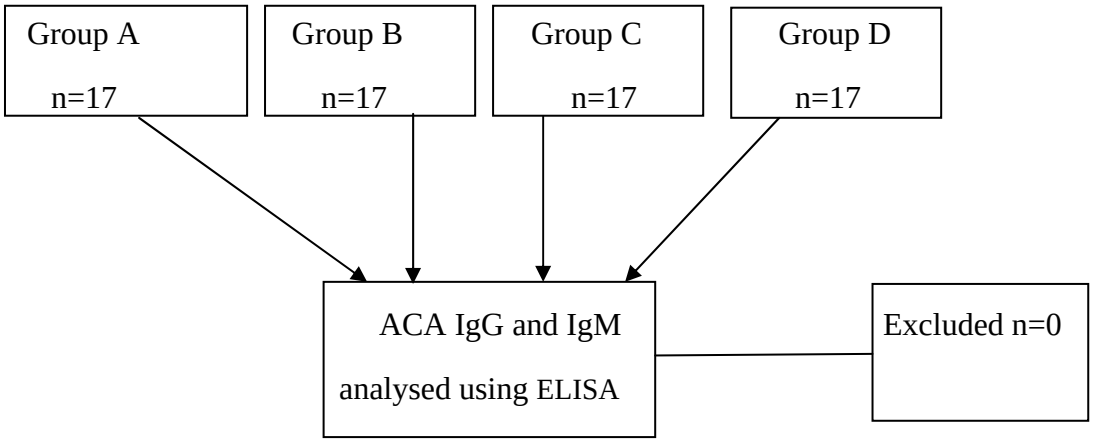
5) Subjects with known history of anti-phospholipid syndrome.

6) Subjects with known history of autoimmune diseases.

7) Smokers, alcoholic and pregnant women.

Study design:

Group A: Healthy controls, Group B: Chronic Periodontitis, Group C: type 2 diabetes mellitus, Group D: Chronic Periodontitis with type 2 Diabetes mellitus.



The nature and design of the study were explained to the patients and consent were obtained for their participation.

Patients included in the study were screened using mouth mirror and periodontal probe. All patients underwent periodontal evaluation, haematological and biochemical analysis. Clinical periodontal parameters were calculated as follows.

Clinical parameters

The following variables were measured.

1. Gingival index

2. Plaque index

3. Oral hygiene index

4. Probing pocket depth

5. Clinical attachment level

1) Gingival index: (Loe H and Silness P. 1963)

The soft tissue surrounding each tooth were divided into 4 gingival scoring units i.e. the distofacial papilla, the facial margin, the mesiofacial papilla and the entire lingual margin. A periodontal probe was used to assess the bleeding of the gingival tissues on probing.

Gingival units were assessed according to the following criteria:

0- Normal gingival

1- Mild inflammation, slight change in colour, no bleeding on palpation.

2- Moderate inflammation, redness, edema & glazing, bleeding on probing.

3- Severe inflammation, marked redness & edema, ulceration, tendency for spontaneous bleeding.

The gingival index score for each of the 4 gingival surfaces was given a score from 0 to 3. The scores around each tooth were totaled and divided by four and the gingival index score for each tooth was obtained.

The scoring criteria are as follows

0.1-1.0 – Mild

1.1-2.0 – Moderate

2.1-3.0 – Severe

2) Plaque index: (Silness P and Loe H. 1964, modified by Loe H. 1967)

0- No plaque in the gingival area.

1- A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be recognized only by running a probe across the tooth surface.

2- Moderate accumulation of soft deposits within the gingival pocket or on the gingival margin & adjacent tooth surface.

3- Abundance of soft matter within the gingival pocket or on the gingival margin & adjacent tooth surface.

The areas examined were the distofacial, facial, mesiofacial and lingual surface using explorer. The plaque score was obtained by totaling the four plaque scores per tooth and then divided by four. The plaque score per person is obtained by adding the plaque score per tooth and dividing by the number of teeth examined.

3) Oral Hygiene index: (Simplified) [Greene and Vermillion 1964]:

The six tooth surfaces were examined. Facial surface of 16,11,26,31 and lingual surface of 36, 46 were examined using an explorer.

Debris index (DI-S)

Dental explorer was placed on the incisal third of the tooth and moved towards the gingival third of the tooth.

The scoring criteria is

0- No debris or stain present

1- Soft tissue covering not more than 1/3rd of the tooth surface or the presence of extrinsic stains without other debris, regardless of surface area covered.

2- Soft debris covering more than 1/3rd but not more than 2/3rd of exposed tooth surface.

3- Soft debris covering more than 2/3rd of the exposed tooth surface.

DI-S score per person is obtained by totaling the debris score per tooth surface divided by number of surfaces examined.

Calculus index: (CI-S)

Assessed by placing a dental explorer into the distal gingival crevice and drawing it subgingivally from distal contact area to the mesial contact area.

0- No calculus present

1- Supragingival calculus covering not more than 1/3rd of the exposed tooth surface

2- Supragingival calculus covering more than 1/3rd of the exposed tooth surface but not more than 2/3rd of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.

3- Supragingival calculus covering more than 2/3rd of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both.

Good – 0.0-0.6

Fair – 0.7-1.8

Poor – 1.9-3.0

The OHI-S score per person is the total of DI-S and CI-S scores per person.

The scoring criteria are as follows

0.0-1.2 - Good

1.3-3.0 – Fair

3.1-6.0 – Poor

4) Probing pocket depth:

The depth of the pocket was measured at four sites per tooth excluding the third molars using periodontal probe. The probe was inserted parallel to the long axis of the tooth gently, until resistance was felt and the readings were recorded to the nearest millimeter from the gingival margin to the base of the pocket.

5) Clinical attachment level:

The level of attachment is the distance between the base of the pocket and Cementoenamel junction (CEJ) or a fixed point. The distance from the CEJ (if CEJ is not detected, the coronal border of the stent was used) to the base of the pocket was measured. The readings were recorded to the nearest millimeter.

PHOTOGRAPHS

Healthy control **(Group A)**

Chronic Periodontitis (Group B)

Type 2 Diabetes Mellitus (Group C)

Type 2 Diabetes Mellitus with Chronic Periodontitis

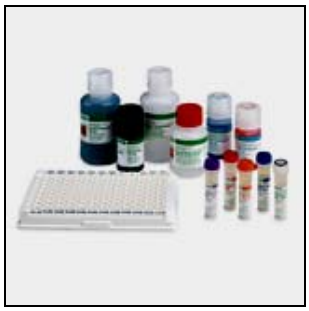
(Group D)

Table top Centrifuge Machine

Serum

ACA IgG test kit

ACA IgM test kit



LABORATORY METHODS

Sample Collection:

2ml venous blood was obtained from each subject in the morning from the ante cubital fossa using 2 ml sterile disposable syringe with 23 gauge needle. The blood was then transferred to empty sterile vacutainer and transported to the clinical laboratory for analysis of ACA IgG and IgM.

Estimation of ACA:

All blood samples were analysed in the same laboratory using enzyme linked immunosorbent assay (ELISA). Standard serum were used to measure the anti-cardiolipin antibody IgG normal range: < 23 GPL units / ml and IgM normal range < 11 MPL units / ml using REALISA anti-cardiolipin IgG and IgM ELISA kit. IgG levels were measured in GPL units and IgM levels were measured in MPL units.

Sample storage:

Blood samples were collected in clean, dry and empty tubes. After separation, the serum samples were stored at -4^0C upto three days.

Principle of the assay:

Purified Cardiolipin antigens are coated on the surface of microwells. Diluted patient serum or plasma, and calibrators, are added to the wells. The Anti-cardiolipin specific antibodies, if present, bind to the antigens.

All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibodies in the sample. The results are read by a microwell reader, and compared in a parallel manner with calibrators.

ELISA READER

Prepare a **1:101** dilution of the patient samples by mixing **5 µl** of the patient sera with **500ul** of Serum Diluent. Incubate **30 minutes** (± 5 min) at room temperature. Wash **4x** with wash buffer. Pipette **100 µl** of Conjugate into microwells. Incubate **30 minutes** (± 5 min) at room temperature.

QUALITATIVE DETERMINATION

$$\frac{\textbf{Abs. of Test Sample}}{\textbf{Abs. of Calibrator D}} \times \textbf{Units of Calibrator D} = \textbf{Units of Test sample}$$

Qualitative results are reported as “positive” or “negative.” Sample results greater than or equal to Calibrator D are considered positive.

Purified Cardiolipin antigens are coated on the surface of microwells. Diluted patient serum or plasma, and calibrators, are added to the wells. The Anti-Cardiolipin specific antibodies, if present, bind to the antigens. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic substrate is added.

ELISA WASHER

The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM specific antibodies in the sample. The results are read by a microwell reader, and compared in a parallel manner with calibrators.

APPENDIX – I

PROFORMA

PATIENT NAME: OP.NO:

AGE: SEX:

ADDRESS: PHONE NO:

CHIEF COMPLAINT:

PAST MEDICAL HISTORY:

KNOWN DIABETIC:

YES ☐ NO ☐

DURATION OF DIABETES:

CONTROLLED/UNCONTROLLED DIABETIC:

HbA_1C :

PRESENT MEDICAL HISTORY:

DRUG HISTORY:

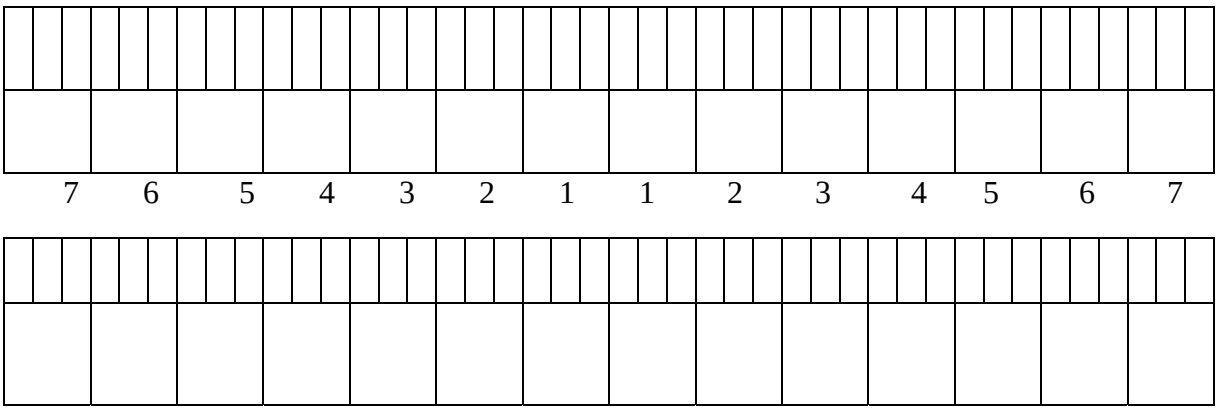
PAST DENTAL HISTORY:

PRESENT DENTAL HISTORY:

INDICES

GINGIVAL INDEX:

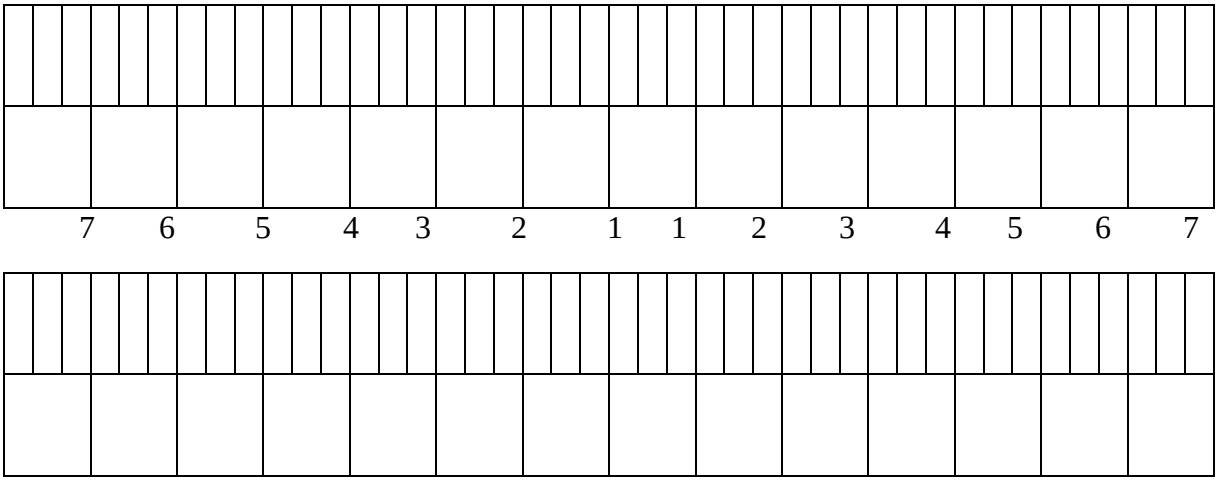
BASELINE: **Maxilla**



Mandible **Score:**

PLAQUE INDEX:

BASELINE: **Maxilla**



Mandible **Score:**

ORAL HYGIENE INDEX (SIMPLIFIED):

DEBRIS INDEX (DI-S):

16	**11**	**26**	**36**	**31**	**46**

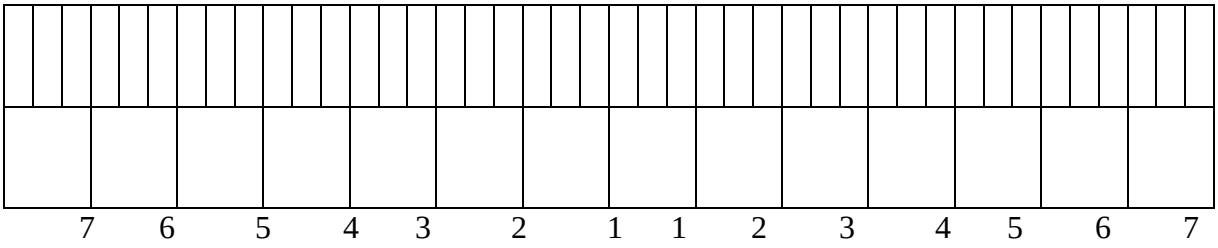
CALCULUS INDEX (CI-S):

OHI-S = CI-S + DI-S

INTERPRETATION:

PROBING POCKET DEPTH:

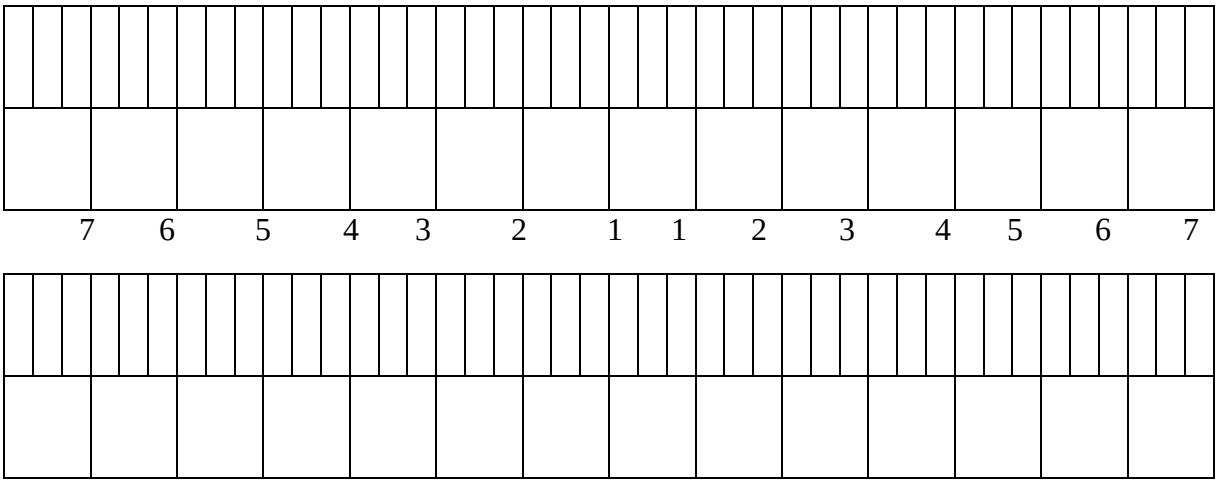
Maxilla



Mandible

CLINICAL ATTACHMENT LEVEL:

Maxilla



Mandible

APPENDIX – 2

ARMAMENTARIUM

1. Mouth mirror
2. Explorer
3. Periodontal probe
4. Sterile cotton rolls
5. Kidney tray
6. Gloves
7. Patient apron
8. Head cap

MATERIALS USED FOR SERUM PREPERATION

1. 10 ml syringe
2. Tourniquet
3. Pipettes
4. Test tubes
5. Centrifuge

INFORMED CONSENT OBTAINED FROM THE PATIENT:

DEPARTMENT OF PERIODONTICS, J.K.K. NATTRAJA DENTAL COLLEGE, KOMARAPALAYAM, NAMAKKAL DISTRICT**.**

PATIENT NAME:

I have been explained about the nature and purpose of the study in which, I have been asked to participate. I understand that I am free to withdraw my consent and discontinue at any time without prejudice to me or effect on my treatment.

I have been given the opportunity to question about the material and study. I have also given the consent for photographs to be taken at the beginning, during and end of the study. I agree to participate in this study.

I hereby give the consent to be included in ***"Evaluation of serum anti-cardiolipin antibody in chronic periodontitis patients with and without diabetes mellitus".***

Station: **SIGNATURE OF PATIENT**

Date: **SIGNATURE OF PROFESSOR**

RESULTS

STATISTICAL ANALYSIS

ANOVA (Analysis of Variance)

Analysis of variance refers to the examination of differences among the samples. It is used to examine the significance of the difference amongst more than two sample means at the same time. The term ANOVA was first proposed by **R.A.Fisher**. The method consists of classifying and cross-classifying statistical results and testing whether the means of specific classification differ significantly.

All the statistical methods were carried out through the **SPSS for Windows (Version 16.0)**

A total of 68 patients were selected and their serum levels of ACA IgG and IgM were analysed using ELISA test and compared using ANOVA.

A total of 68 patients with 17 patients in each group were selected in this present study. The biochemical parameters of ACA IgG were shown in the Table and Graph No. 1. The mean serum levels of ACA IgG of group A was 3.47 ±0.36. The mean serum levels of ACA IgG of group B was 5.33±0.523 and group C was 4.35±1.12 and group D was 6.13±0.61. No patient had ACA IgG positivity.

The biochemical parameters of ACA IgM were shown in the Table and Graph No.2. The mean serum levels of ACA IgM of group A was 3.26±0.70. The mean serum levels of ACA IgM of group B was 1.96±0.90 and group C was 3.47±0.06 and group D was 2.32±0.81. Only one patient had ACA IgM (>11 MPL units/ml) positivity.

There was a significant difference ($P<0.05$) in the serum ACA IgG between group A (3.47 ±0.36) and group B (5.33±0.523) as shown in the Table and Graph No.3. There was a statistically significant difference between group A (3.47 ±0.36) and group C (4.35±1.12). and between group A (3.47 ±0.36) and group D (6.13±0.61) as shown in the Table and Graph No.3.

There was a statistically significant difference ($P<0.05$) in the serum ACA IgM between group A (3.26±0.70) and group B (1.96±0.90) as shown in the Table and Graph No.4. There was no statistically significant difference ($P>0.05$) between group A (3.26±0.70) and group C (3.47±0.06) and between group A (3.26±0.70) and group D ((2.32±0.81) as shown in the Table and Graph No.4.

The intragroup comparison of serum ACA IgG and IgM of group A shows a statistically significant difference ($P<0.05$) as shown in the Table and Graph No.5. The intragroup comparison of ACA IgG and IgM of group B, C, D showed a statistically significant difference ($P<0.05$) as shown in the Table and Graph No.5.

TABLES

TABLE – 1

Mean ACA IgG titer of all study groups

S.No	Parameters	ACA IgG titer
1	Group A	3.47±0.36
2	Group B	5.33±0.53
3	Group C	4.35±1.12
4	Group D	6.13±0.61

ACA – Anti-Cardiolipin Antibody

TABLE – 2

Mean ACA IgM titer of all study groups

S.No	Parameters	ACA IgM titer
1	Group A	3.26±0.70
2	Group B	1.96±0.90
3	Group C	3.47±0.06
4	Group D	2.32±0.81

TABLE – 3

Comparison of mean ACA IgG of Group A with other three groups

S.No	Parameters	ACA IgG titer	P value < 0.05
1	Group A vs Group B	3.47±0.36 Vs 5.33±0.53	0.029*****
2	Group A vs Group C	3.47±0.36 Vs 4.35±1.12	0.000*****
3	Group A vs Group D	3.47±0.36 Vs 6.13±0.61	0.000*****

Significant***** (P<0.05)

TABLE – 4

Comparison of mean ACA IgM of Group A with other three groups

S. No	Parameters	ACA IgM titer	P value < 0.05
1	Group A Vs Group B	3.26±0.70 Vs 1.96±0.90	0.037*
2	Group A Vs Group C	3.26±0.70 Vs 3.47±0.06	0.370
3	Group A Vs Group D	3.26 ±0.70 Vs 2.32±0.81	0.490

Significant* (P<0.05)

TABLE – 5

Intragroup comparison of ACA IgG and IgM of all study groups

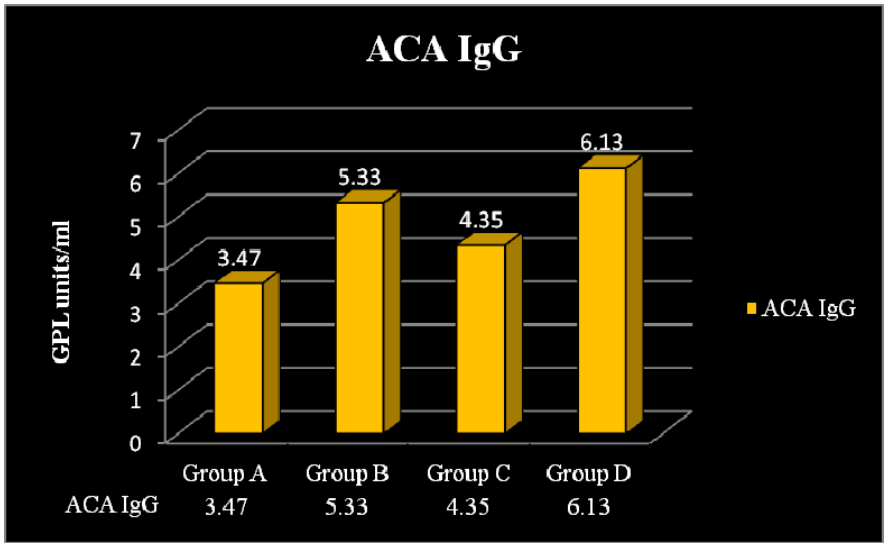
S.No	Parameters	ACA IgG titer	ACA IgM titer	P value < 0.05
1	Group A	3.47 ±0.36	3.26±0.70	0.0001*
2	Group B	5.33±0.53	1.96±0.9	0.0390*
3	Group C	4.35±1.12	3.47±0.06	0.0001*
4	Group D	6.13±0.61	2.32±0.81	0.0001*

Significant * (P<0.05)

GRAPHS

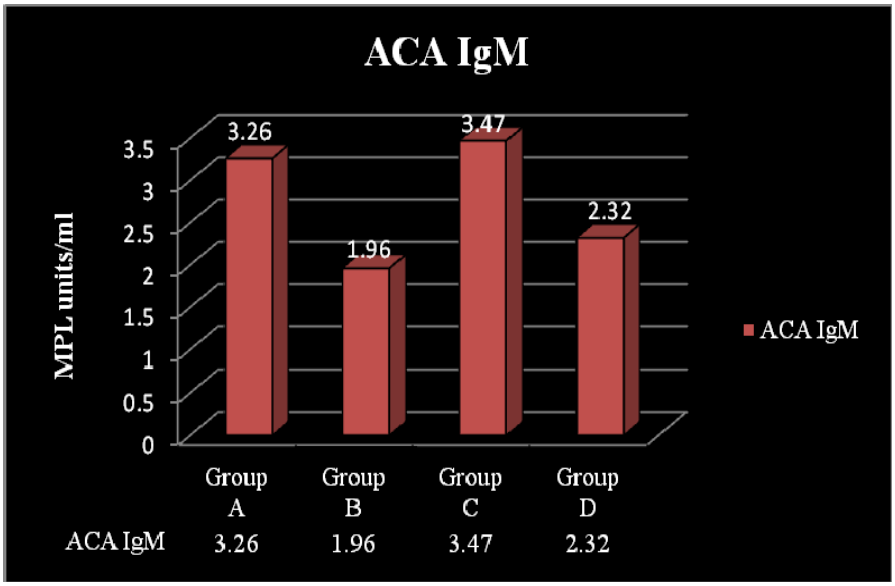
GRAPH - 1

Comparison of mean ACA IgG of all study groups



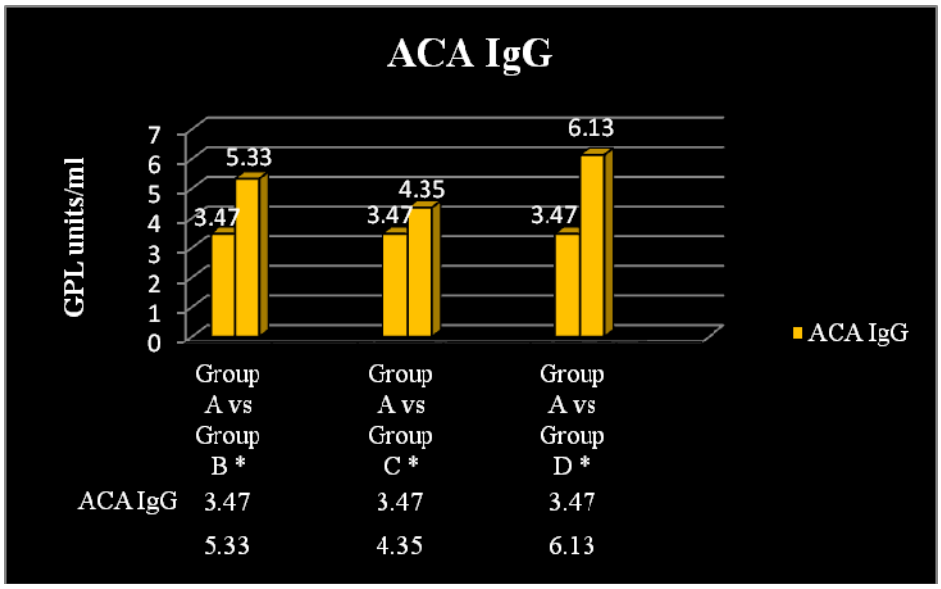
GRAPH- 2

Comparison of mean ACA IgM of all study groups



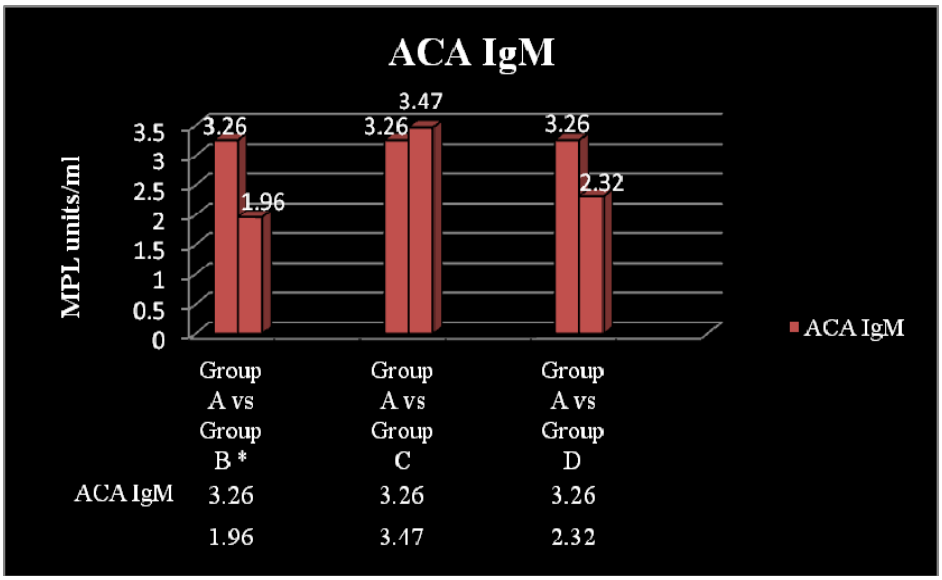
GRAPH – 3

Comparison of ACA IgG of group A with other three groups



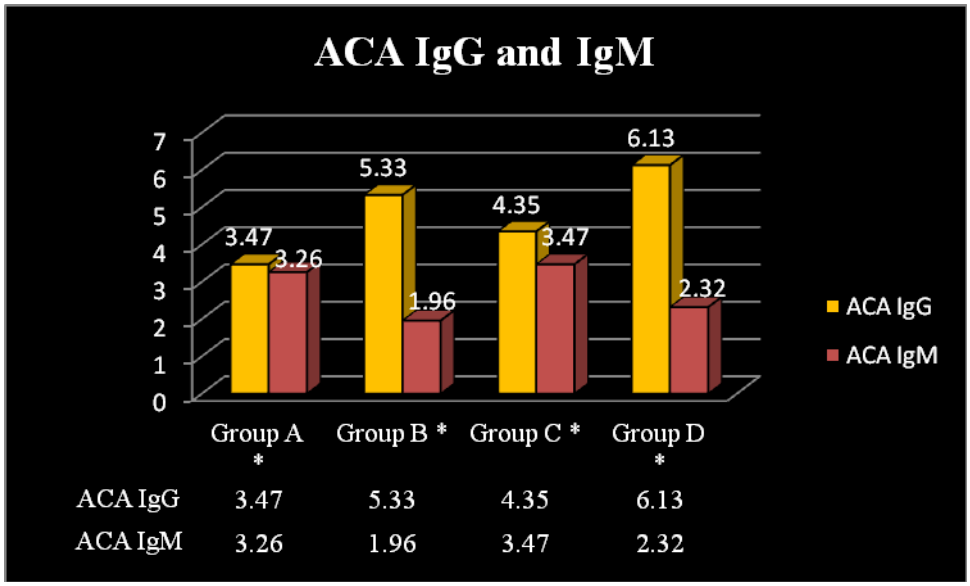
GRAPH –4

Comparison of ACA IgM of Group A with other three groups



GRAPH - 5

Comparison of mean ACA IgG and IgM of all study groups



DISCUSSION

Diabetes Mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action or both[3].

DM is proved to be the risk factor for periodontitis and cardiovascular diseases. Type 2 DM patients who have uncontrolled blood glucose levels are more prone for periodontal destruction. It is proved that periodontal health improved the metabolic control of type 2 DM patients.

Periodontitis and cardiovascular diseases are closely related as both are chronic inflammatory disease and atherosclerosis which is a focal thickening of the arterial intima and media is the major cause of death in all cardiac patients.

Cardiolipin one of the phospholipid present in the inner mitochondrial membrane elicits antibody response in DM patients due to mitochondrial destruction[1]. It is also elevated in acute myocardial infarction, other systemic infections and also in periodontitis[3].

Anti-cardiolipin antibodies are autoantibodies directed against cardiolipin, a phospholipid. These antibody levels are elevated in periodontitis[3], diabetes[13], infections and cardiovascular diseases[14]. The ACA IgG and IgM levels are evaluated in four different groups namely: Group A: Healthy controls, Group B: Chronic Periodontitis, Group C: Type 2 Diabetes Mellitus, Group D: Chronic Periodontitis with type 2 Diabetes Mellitus.

When IgG ACA levels were compared between group A (3.47±0.36) and group B (5.33±0.53), the levels were significantly elevated in group B (P<0.05). The same results were obtained by *Schenkein HA et al.*[85] who found that the prevalence of ACA were higher in chronic periodontitis patients when compared with healthy patients as the severity and the extent of inflammation were more in periodontitis patients.

When IgG ACA levels were compared between group A (3.47±0.36) and Group C (4.35±1.12), the levels were significantly elevated in group C (P<0.05). The results obtained in this present study were in accordance with the study done by *Romero J et al.*[13] who found that low ACA IgG and IgM may occur in type 2 diabetic patients.

When IgG ACA levels were compared between group A (3.47±0.36) and group D (6.13±0.61), the levels were significantly higher in group D (P<0.05). The probable reason may be due to the presence of both systemic (DM) and local infection (Periodontitis) which together caused this elevation of ACA IgG levels. The elevated IgG titer leads to systemic thrombotic events as shown by *Kalra S et al.*[7]

When IgM ACA levels are compared between group A (3.26±0.70) and group B (1.96±0.90), the levels were significantly lower in group B (P < 0.05). The similar type of results were obtained by *Schenkein HA et al.*[84] who found that ACA levels were elevated in Periodontitis patients due to the presence of phosphorylcholine containing antigen in oral species compared to the healthy patients.

When IgM ACA levels are compared between group A (3.26±0.70) and group C (3.47±0.06), the levels were not significantly lower in group C (P>0.05).

In this present study the IgM ACA levels when compared between group A (3.26±0.70) and group D (2.32±0.81), the levels were not significantly lower in group D (P>0.05). This could be probably due to the reason that IgM had inverse association with the severity of cardiac events according to *Bili et al.*[81] who found that elevated IgM were not associated with any systemic complications. According, to the authors IgM ACA represent protective natural autoantibodies or they have rheumatoid factor activity and thus play a beneficial role in immune homeostasis. Therefore, the ACA IgM levels which were high in Group A proves that these patients are not prone for cardiac events but in group D more prone for cardiac events.

The above results indirectly proves the relationship that group D who had high levels of IgG and low levels of IgM are more prone for cardiac diseases like atherosclerosis, stroke and thrombosis. Therefore, chronic periodontitis may be one of the risk factor for elevating the ACA levels in these groups of patients.

Based on the above results, Chronic Periodontitis not only causes lipid abnormality and hyperglycemia but also elevates anti-cardiolipin antibody levels in diabetic patients which was proved in this present study.

Generally, it is well known that ACA assay when employed in the cases of recurrent abortions, CHD, Stroke, Syphilis, Atherosclerosis it is found to be elevated. Likewise, in this present study when ACA assay were employed in type 2 Diabetic patients, it were found to be in low titer, whereas in Chronic Periodontitis patients it were found to be moderately increased.

But in Chronic Periodontitis with type 2 Diabetes Mellitus group, ACA levels were significantly elevated ($P<0.05$) compared to healthy controls. Ultimately the contribution of Chronic Periodontitis decides the increase in the levels of anticardiolipin antibodies in diabetic patients.

However, the limitations of this present study were small sample size, no follow up of cases and there was no periodontal treatment done to find out the influence of periodontal treatment on the levels of anticardiolipin antibodies.

SUMMARY AND CONCLUSION

The aim of the present study was to evaluate the ACA levels in four different groups and also to find out whether Chronic Periodontitis has any role to play and influence on the levels of ACA in diabetic patients.

The anti-cardiolipin antibody levels (IgG and IgM) were measured using Enzyme Linked Immuno Sorbent Assay (ELISA). The levels were subjected to statistical analysis and compared using ANOVA (analysis of variance).

From the results obtained, the following conclusions were arrived:

***** Chronic Periodontitis with type 2 Diabetes Mellitus group had elevated levels of IgG ACA compared to that of Chronic Periodontitis group, Type 2 Diabetes Mellitus group and Healthy controls. Therefore, Chronic Periodontitis may act as an additional risk factor in diabetic patients to increase the ACA levels.

***** The IgM ACA levels were low in Chronic Periodontitis with type 2 Diabetes Mellitus group which proves that these groups are more prone for cardiac thrombosis. IgM have inverse association with cardiac events[17].

In the light of the facts mentioned above, Chronic Periodontitis can be considered as a risk factor for diabetic patients to develop cardiovascular complications.

The results of the present study have proved that a strong and definite association exists between periodontal diseases and systemic diseases. Periodontal disease will contribute directly to the pathogenesis of atherosclerosis by providing persistent bacterial challenges.

The anticardiolipin antibody assay employed in this study can be utilized as a biomarker for assessing the risk of the patients (in general) like Diabetes Mellitus, Atherosclerosis, Stroke and also the risk of Periodontitis patients (in particular) for developing systemic diseases.

Any research study is bound to be questioned in respect of various factors such as anamolies, presumptions, leniences, environmental differences, etc., Similarly, this study also has some limitations. In order to overcome those limitations to some extent, a large sample size with regular follow up accompanying periodontal treatment can better discover the relationship among periodontitis, anticardiolipin antibody and systemic diseases.

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